PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Developmental Programmes of Growth and Survival in Early Sensory Neurons

Alun M. Davies and Kristine S. Vogel

Phil. Trans. R. Soc. Lond. B 1991 **331**, 259-262
doi: 10.1098/rstb.1991.0014

Developmental programmes of growth and survival in early sensory neurons

ALUN M. DAVIES AND KRISTINE S. VOGEL

Department of Anatomy, St. George's Hospital Medical School, Cranmer Terrace, Tooting, London SW17 0RE, U.K.

SUMMARY

In the developing vertebrate nervous system the survival of neurons becomes dependent on the supply of a neurotrophic factor from their targets when their axons reach these targets. To determine how the onset of neurotrophic factor dependency is coordinated with the arrival of axons in the target field, we have studied the growth and survival of four populations of cranial sensory neurons whose axons have markedly different distances to grow to reach their targets. Axonal growth rate both *in vivo* and *in vitro* is related to target distance; neurons with more distant targets grow faster. The onset trophic factor dependency in culture is also related to target distance; neurons with more distant targets survive longer before becoming trophic factor dependent. These data suggest that programmes of growth and survival in early neurons play an important role in coordinating the timing of trophic interactions in the developing nervous system.

1. INTRODUCTION

Neurons are generated in excess in the developing vertebrate nervous system. Superfluous and inappropriately connected neurons are eliminated in a phase of cell death which begins shortly after neurons innervate their target fields (Oppenheim 1981). It is now widely accepted that the target field plays an important role in regulating the number of neurons that survive by its production of a limited quantity of a neurotrophic factor which the innervating neurons require for their survival. Neurons that are able to procure an adequate supply of this factor survive whereas neurons that are unsuccessful in this competition die. Evidence for this 'neurotrophic hypothesis' has come principally from work on nerve growth factor (NGF), the first neurotrophic factor to be identified and characterized (Levi-Montalcini & Angeletti 1968; Thoenen & Barde 1980; Davies 1988*a*, *b*; Barde 1989).

The most important direct evidence is the finding that developing neurons whose survival is promoted by NGF *in vitro*, namely sympathetic and certain kinds of sensory neurons, are also dependent on NGF *in vivo*. Anti-NGF antibodies eliminate these neurons during the phase of target field innervation whereas exogenous NGF rescues neurons that would otherwise die. Considerable indirect support comes from studies of the synthesis, uptake and transport of NGF. The target fields of NGF-dependent neurons contain trace quantities of NGF in proportion to their innervation density. Specific cell-surface receptors mediate the uptake of NGF by nerve fibres in the target field. Fast axonal transport conveys the internalized receptor–ligand complex from the target field to the cell bodies of the innervating neurons where it exerts its survival-promoting effects. Comparable findings in similar *in vivo* and *in vitro* studies of the homologous neurotrophic factor, brain-derived neurotrophic factor (BDNF), have further strengthened this hypothesis and shown its generality (Barde 1988, 1989; Davies 1988*b*).

Studies of the development of the embryonic mouse trigeminal system have revealed that the onset of the trophic interactions between neurons and their targets is closely coordinated with the onset of target field innervation. Measurements of the levels of NGF and NGF mRNA in the developing peripheral target field of the trigeminal ganglion have shown that NGF synthesis commences with the onset of innervation (Davies *et al.* 1987). When the trigeminal ganglion neurons are cultured before their axons have reached their targets they survive independently of NGF, but when cultured after their axons have reached their targets these neurons die unless NGF is present in the culture medium (Davies & Lumsden 1984). The close temporal relationship between the onset of NGF-dependency and the arrival of axons in the target field is consistent with the finding that NGF receptors are first detected on trigeminal neurons at this stage of their development (Davies *et al.* 1987) and that the level of NGF receptor mRNA increases markedly at this stage (Wyatt *et al.* 1990). *In vitro* studies of developing avian retinal ganglion cells have likewise shown that these neurons become dependent on BDNF for survival at the stage their axons reach their targets (Rodriguez-Tebar *et al.* 1989). The acquisition of neurotrophic factor dependency by these neurons and early sensory neurons occurs *in vitro* independently of target cell encounter (Davies & Lumsden 1984; Ernsberger & Rohrer 1988; Rodriguez-Tebar *et al.* 1989).

Because the distance axons have to grow to reach their targets varies from one population of neurons to another, there must be mechanisms to ensure that the

Phil. Trans. R. Soc. Lond. B (1991) **331**, 259–262
Printed in Great Britain

onset of neurotrophic factor dependency is coordinated with the arrival of axons in the target field. Either the target provides the innervating neurons with a signal that initiates dependency or the neurons possess a developmental programme that initiates dependency at the appropriate time. In addition to target distance, the timing of target field innervation is also dependent on the rate at which axons grow to reach their targets and this may also be an independently regulated parameter that plays a role in coordinating trophic interactions in the developing nervous system.

To investigate the nature of the mechanisms that control the timing of trophic interactions in the developing nervous system we have studied the survival and growth of embryonic chick cranial sensory neurons in culture. Our findings suggest that both the target field and developmental programmes of growth and survival in the neurons play a role in initiating neurotrophic factor dependency at the correct time.

2. EXPERIMENTAL ADVANTAGES OF CRANIAL SENSORY NEURONS

Cranial sensory neurons are ideal candidates for a comparative study of axonal growth rate and neuronal survival because they are located in discrete ganglia that can be dissected from the embryo at the stage when their peripheral and central axons are growing to their targets in the periphery and central nervous system (CNS). The distance these axons have to grow to reach their targets is different for each ganglion and ranges from several hundred microns in the case of the vestibular ganglion which innervates targets in its immediate vicinity to several thousand microns in the case of the nodose ganglion which innervates distant targets in the thorax and abdomen.

Figure 1. Camera lucida drawing of a stage 24 wholemount silver-stained chick embryo showing the location of the vestibular (V), geniculate (G), petrosal (P) and nodose (N) ganglia. The major peripheral and central nerve branches of these ganglia are also shown.

To permit comparison of axonal growth and neuronal survival of different neurons at the same stage of development, we restricted our study to the vestibular, geniculate, petrosal and nodose ganglia whose neurons are born over the same period of development (between 2 and 5 days *in ovo* (D'Amico-Martel 1982). These ganglia are also the only ones in which the neurons are derived exclusively from ectodermal placodes (D'Amico-Martel & Noden 1983), and in this respect they share a similar ontogeny. The distance between the CNS and peripheral targets fields of these ganglia increases from vestibular through geniculate and petrosal to nodose (figure 1).

3. AXONAL GROWTH RATE

Estimates of the rate at which vestibular, geniculate, petrosal and nodose axons grow to their targets *in vivo* were made from measurements of nerve lengths in a staged series of silver-stained embryo wholemounts (Davies 1989). Vestibular neurons have the slowest growth rate, geniculate and petrosal neurons are between two and threefold faster and nodose neurons are fivefold faster. This order accords with the distances that the neurons of these ganglia have to grow to reach their targets in the embryo; the further the targets, the faster the neurons grow.

To determine if these differences in growth rate are intrinsic properties of the neurons or are due to differences in the environment through which their axons grow *in vivo*, neurite growth rate was studied in low-density dissociated cultures of these neurons grown on a laminin substratum (Davies 1989). Cultures were established at stages 19, 20 and 22, that is early during the period neuroblasts undergo their terminal division. In these cultures neurons exhibited a bipolar morphology with fine, unbranched neurites growing in opposite directions from small, spindle-shaped cell bodies. From serial measurements of neurite length made during the first 18 h in culture it was determined that the neurons of each ganglion grow distinct rates which are similar to those observed *in vivo*. These distinct differences in neurite growth rate were not due to substances released into the culture medium by other cells because similar results were observed in single-cell cultures of these neurons set up in multiwell plates. These results suggest that neurite growth rate is an intrinsically regulated characteristic of early neurons.

4. TIMING AND REGULATION OF NEUROTROPHIC FACTOR DEPENDENCY

To determine if the duration of neurotrophic factor independent survival differs for early vestibular, geniculate, petrosal and nodose neurons, low-density, dissociated cultures of these neurons were established from stage 19 or stage 20 embryos and grown in the absence of neurotrophic factors (K. S. Vogel &

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

A. M. Davies, unpublished results). After 12 h incubation a cohort of neurons in each culture was identified and the number of neurons remaining in each cohort was subsequently monitored at 12-h intervals. In these cultures distinct differences in the rate at which neurons die were observed. Vestibular neurons die most rapidly, nodose neurons die much more slowly and geniculate and petrosal neurons die at an intermediate rate. These differences in the duration of neurotrophic factor-independent survival are also related to target distance; neurons with more distant targets survive longer.

The survival of sensory neurons during the stage of target field innervation is regulated by two different neurotrophic factors derived from their peripheral and central target fields, respectively (Davies *et al.* 1986*a*). At this stage vestibular, geniculate, petrosal and nodose neurons are dependent on BDNF from their central targets (Davies *et al.* 1986*b*). To determine if developing cranial sensory neurons regulate the onset of BDNF-dependency in accordance with target distance, the survival of stage 19/20 neurons has been monitored in very low-density dissociated cultures in the presence and absence of BDNF (K. S. Vogel and A. M. Davies, unpublished results). In these cultures sensory neurons differ in the time at which BDNF first affects their survival. For the first 24 h in culture BDNF has no effect on neuronal survival. By 36 h vestibular neurons have started to respond to BDNF with enhanced survival. Geniculate neurons are the next to respond at 48 h. Petrosal neurons start responding by 60 h and BDNF has no effect on the survival of nodose neurons until 72–84 h *in vitro*. This order in the onset of responsiveness of cranial sensory neurons to BDNF is again related to target distance; neurons with more distant targets survive longer in culture before becoming responsive to BDNF. Furthermore, the order in the onset of BDNF responsiveness in these neurons is also the same order in which their central axons reach the brainstem where BDNF is synthesized (K. S. Vogel and A. M. Davies, unpublished results). These observations suggest that the onset of neurotrophic factor dependency in developing sensory neurons is controlled at least in part by a developmental clock in these neurons.

To determine if this clock is influenced by BDNF itself, the effects of briefly exposing early nodose neurons to BDNF after various times in culture has been studied. When neurons are exposed to BDNF from 24–48 h in culture the neurons die at the same rate as in controls, but when exposed to BDNF from 48–72 h the neurons subsequently die at a much faster rate than in controls. This suggests that exposure to BDNF shortly before the neurons start becoming dependent on BDNF for survival in culture accelerates the onset of dependency.

5. ONTOGENY OF PROGRAMMES OF GROWTH AND SURVIVAL

To determine when and how differences in axonal growth rate and the timing trophic factor dependency become established it is necessary to consider the ontogeny of the vestibular, geniculate, petrosal and nodose ganglia. The cells of these ganglia originate from two sources: the neurons are derived from regional thickenings of the head ectoderm termed placodes and the supporting cells are derived from the neural crest (D'Amico-Martel & Noden 1983). Differences in neuronal growth and survival may either arise in placodes by the interpretation of positional information distributed along the body axis or may be conferred on placodal cells by the neural crest cells that migrate past them or associate with them in the developing ganglia. That neural crest cells from different axial levels possess different patterning information before migration is shown by the finding that replacement of presumptive second branchial arch neural crest with presumptive first arch neural crest results in the formation of ectopic first arch musculoskeletal components (Noden 1983).

Preliminary studies have been undertaken of the survival of neurons that differentiate in dissociated cell cultures of the otic vesicle and third epibranchial placode at the stage when cells are budding off these structures to form the neurons of the vestibular and nodose ganglia, respectively (K. S. Vogel & A. M. Davies, unpublished results). In these cultures presumptive vestibular neurons die at a considerably faster rate than presumptive nodose neurons, that is, the characteristic differences neuronal death rate that are observed in cultures of early vestibular and nodose ganglion neurons are also observed in neurons that differentiate from the corresponding progenitor cells in culture. This finding suggests that the clock which regulates the duration of neurotrophic factor-independent survival is already set in the vestibular and nodose neuron progenitor cells before overt neuronal differentiation. Furthermore, it also eliminates the possibility that the differences in neuronal death rate observed in dissociated cultures of vestibular and nodose ganglia set up at stage 19/20 is not because of the differences in the proportion of these neurons that may have already innervated their targets in the hindbrain.

6. DEVELOPMENTAL SIGNIFICANCE

Our studies suggest that developing sensory neurons use two mechanisms for coordinating the onset of neurotrophic factor dependency with the arrival of their axons in the target field. First, the axons of neurons with more distant targets grow faster to reach these targets. Second, neurons with more distant targets survive longer before they become dependent on neurotrophic factors for survival. These mechanisms may ensure that developing neurons that are born at different distances from their targets do not become dependent on neurotrophic factors until their axons have reached their targets and gained access to these factors. The acceleration of BDNF responsiveness by exposure to BDNF near the end of the phase of neurotrophic factor-independent survival raises the possibility that the target field may also play a role in controlling the onset of neurotrophic factor dependency.

This work was supported by a grant from The Wellcome Trust.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

REFERENCES

Barde, Y.-A. 1988 What, if anything, is a neurotrophic factor? *Trends Neurosci.* **11**, 343–346.

Barde, Y.-A. 1989 Trophic factors and neuronal survival. *Neuron* **2**, 1525–1534.

D'Amico-Martel, A. 1982 Temporal patterns of neurogenesis in avian cranial sensory and autonomic ganglia. *Am. J. Anat.* **163**, 351–372.

D'Amico-Martel, A. & Noden, D. M. 1983 Contributions of placodal and neural crest cells to avian cranial peripheral ganglia. *Am. J. Anat.* **166**, 445–468.

Davies, A. M. 1988*a* Role of neurotrophic factors in development. *Trends Genetics* **4**, 139–143.

Davies, A. M. 1988*b* The emerging generality of the neurotrophic hypothesis. *Trends Neurosci.* **11**, 243–244.

Davies, A. M. 1989 Intrinsic differences in the growth rate of early nerve fibres related to target distance. *Nature, Lond.* **337**, 553–555.

Davies, A. M., Bandtlow, C., Heuman, R., Korsching, S., Rohrer, H. & Thoenen, H. 1987 Timing and site of nerve growth factor synthesis in developing skin in relation to innervation and expression of the receptor. *Nature, Lond.* **326**, 353–358.

Davies, A. M. & Lumsden, A. G. S. 1984 Relation of target encounter and neuronal death to nerve growth factor responsiveness in the developing mouse trigeminal ganglion. *J. comp. Neurol.* **253**, 13–24.

Davies, A. M., Thoenen, H. & Barde, Y.-A. 1986*a* Different factors from the central nervous system and periphery regulate the survival of sensory neurones. *Nature, Lond.* **319**, 497–499.

Davies, A. M., Thoenen, H. & Barde, Y. A. 1986*b* The response of chick sensory neurons to brain-derived neurotrophic factor. *J. Neurosci.* **6**, 1897–1904.

Ernsberger, U. & Rohrer, H. 1988 Neuronal precursor cells in chick dorsal root ganglia: Differentiation and survival *in vitro*. *Devl. Biol.* **126**, 420–432.

Levi-Montalcini, R. & Angeletti, P. U. 1968 Nerve growth factor. *Physiol. Rev.* **48**, 534–569.

Noden, D. M. 1983 The role of neural crest in patterning of avian cranial skeletal, connective, and muscle tissues. *Devl. Biol.* **96**, 144–165.

Oppenheim, R. W. 1981 Neuronal death and some related phenomena during neurogenesis: a selective historical review and progress report. In *Studies in developmental Biology* (ed. W. M. Cowan), pp. 74–133. Oxford University Press.

Rodriguez-Tebar, A., Jeffrey, P. L., Thoenen, H. & Barde, Y.-A. 1989 The survival of chick retinal ganglion cells in response to brain-derived neurotrophic factor depends on their embryonic age. *Devl. Biol.* **136**, 296–303.

Thoenen, H. & Barde, Y.-A. 1980 Physiology of nerve growth factor. *Ann. Rev. Physiol.* **60**, 284–335.

Wyatt, S., Shooter, E. M. & Davies, A. M. 1990 Expression of the NGF receptor gene in sensory neurons and their cutaneous targets prior to and during innervation. *Neuron* **4**, 421–427.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES